An Amperometric Detection in High-Performance Liquid Chromatography for Monitoring Plasma Free Fatty Acids

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Introduction

Plasma free fatty acids (FFAs) are indicator substances in lipid, glucose and hormone metabolism and are useful in the diagnosis of various diseases such as diabetes mellitus and acute pancreatitis. The determination of FFAs in plasma and serum has been done by gas chromatography (GC) or GC-mass spectrometry, and high-performance liquid chromatography (HPLC). However, these methods have only limited application in disease diagnosis. The cleanup and derivatization of FFAs before separation are complicated and time-consuming.

We developed a new method for determining acids by means of voltammetric reduction of quinone [1,2]. In the present study, an HPLC system with electrochemical detection (ECD) was fabricated for determining FFAs in human plasma, since the acid content can be monitored by measuring the current signal of the reduction peak of quinone caused by the presence of acid. Because of the simple and rapid procedure without the derivatization, the present HPLC-ECD was evaluated as an effective method capable of monitoring the changes in plasma FFAs accompanying the changes of blood glucose level.

Experimental

Separation of FFAs was done by the HPLC-ECD using an octadecylsilica (ODS) column and a glassy carbon working electrode. An ethanol-acetonitrile (20:80) mixture and that containing 6 mM 2-methyl-1,4-naphthoquinone and 76 mM $LiClO_4$ served as a mobile phase and a quinone solution, respectively. Both solutions were made to flow at 1.1 mL/min. To prepare the FFA sample solution, FFAs extracted from 10 µL of control serum or human plasma with diethyl ether were dissolved in the ethanol-acetonitrile (20:80) mixture (40 µL). A 20 µL of the sample solution was injected into the column, and the eluate was mixed with the quinone solution and each fatty acid was detected with ECD at –415 mV vs. SCE.

Result and Discussion

A typical chromatogram for a standard acid mixture of myristic , palmitic, palmitoleic, margaric, stearic, oleic, linoleic and arachidonic acids at 1 nmol each is shown in Fig. 1A. The acids were well separated within 10 min. Peak height was linearly related to the amount of acid injected, ranging from 50 to 1600 pmol ($r > 0.997$) in all cases. Relative standard deviation (RSD) of the acids at 200 pmol was less than 1.5 % ($n = 10$), and the acid detection limit ($S/N = 3$) for a single injection was 50 pmol. The chromatogram obtained following the injection of the sample solution prepared from control serum is shown in Fig. 1B. Based on the peak heights, FFA content was determined with the RSD of less than 5 % ($n = 10$).

Plasma FFAs were determined using blood from two volunteer subjects. The recovery of FFAs was tested using the plasma spiked with acid standards. The results in all cases were 92 ~ 102 % with the RSD of less than 4.5 % ($n = 5$). The present method required small sample amounts (10 µL) of plasma and a short time for the blood pretreatment, indicating the present method to be promising for monitoring plasma FFAs.

The changes in plasma FFAs accompanying those in blood glucose level were monitored before and after meal ingestion by this method. Blood glucose was determined froma 5µL blood specimen using a pocket size glucose meter. The results are shown in Fig. 2, in which FFAs decrease rapidly in contrast to the increase in glucose level following the meal ingestion. Thereafter, the reduction of glucose and the increase of plasma FFAs occur.

The present method is thus shown to have potential for clinical applications.

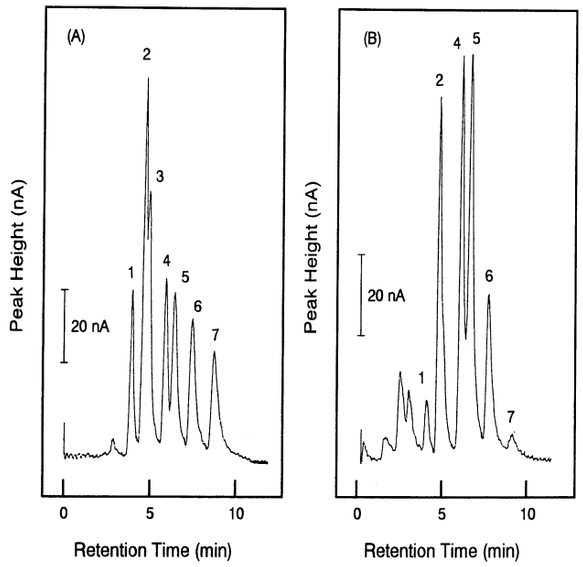

Fig. 1. Chromatograms of FFAs obtained from a standard acid mixture (A) and control serum (B). Peaks are corresponding to: 1, arachidonic acid; 2, palmitoleic and linoleic acids; 3, myristic acid; 4, oleic acid; 5, palmitic acid; 6, margaric acid (internal standard); 7, stearic acid.

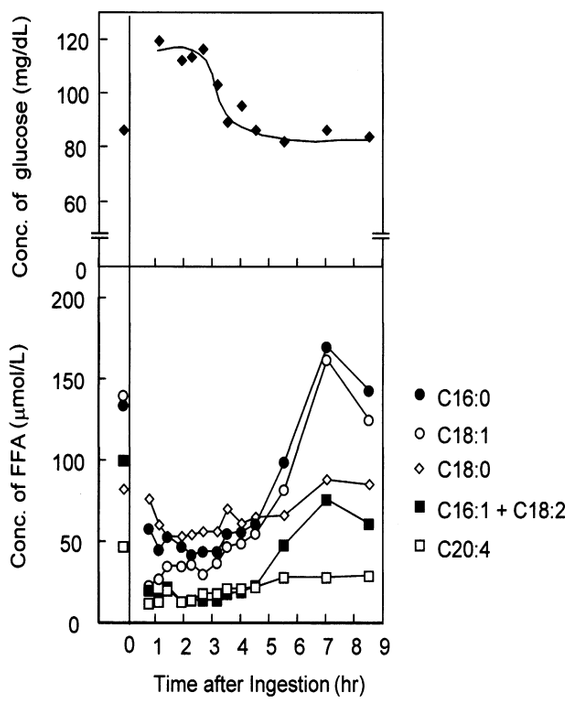

Fig. 2. Changes in plasma FFAs (lower) and blood glucose (upper) levels. Subject: a 50-year-old female.

References

1. K. Takamura, T. Fuse, K. Arai and F. Kusu, *J. Electroanal. Chem.*, **468** (1999) 53.
2. T. Fuse, F. Kusu and K. Takamura, *J. Chromatogr. A*, **764** (1997) 177.